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Inoculation of Strawberries with AM Fungi Produced On-Farm Increased Yield

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ABSTRACT

Inoculation of plants with arbuscular mycorrhizal [AM] fungi has the potential to increase or maintain yields and allow for reduced fertilizer and pesticide application, thereby enhancing agricultural sustainability. Strawberry plants (*Fragaria x ananassa* Duch. cv. Chandler) were inoculated prior to outplanting with a mixed species inoculum of AM fungi. The inoculum was produced on-the-farm in 2003 in a mixture of compost and vermiculite with bahiagrass (*Paspalum notatum* Flugge) as host plants. Plants were outplanted into raised black plastic beds on 30 June 2004 and harvested 6–25 June 2005. Inoculation with AM fungi increased yield 17% over uninoculated controls, 5.5 vs. 4.7 kg per ten plant sampling unit, respectively. Inoculation had no significant effect on whole season mean fruit weight, indicating an average increase of 3.6 fruit per plant for inoculated plants over uninoculated plants. Utilization of AM fungus inoculum produced on-farm as an amendment to horticultural potting media for the production of seedlings later outplanted has the potential for significant increases in crop yields.

INTRODUCTION

Arbuscular mycorrhizal [AM] fungi are obligate symbiotic soil fungi which colonize the roots of the majority of crop plants. Many benefits to the plant have been ascribed to being in symbiosis with AM fungi. The most frequently reported benefit is enhanced uptake of immobile nutrients, notably P, from the soil solution (Hayman & Mosse, 1972; Bolan, 1991). The extraradical

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mycelium of the fungi act as extensions of the root system by exploring a larger volume of soil for immobile nutrients than is accessible to the plant's root hairs (Rhodes & Gerdemann, 1975; Li *et al.*, 1991). Other benefits to the plant include increased resistance to pathogens and water stress (Augé, 2000; Harrier & Watson, 2004) and the indirect benefits the plant receives due to the fungus' role in stabilizing soil aggregates (Rillig & Mummey, 2006). Utilization of AM fungi in agriculture has the potential to decrease synthetic chemical inputs and produce economic and environmental benefits.

A farmer typically has two opportunities to inoculate a crop with AM fungi. The plant can be inoculated in the field, whether at seeding or during outplanting. This leaves the inoculated isolates of AM fungi to compete with the indigenous population of AM fungi for initial colonization of the roots. Even so, field inoculation can successfully enhance mycorrhiza formation on roots if the native population has been negatively impacted by management practices, e.g. fumigation (Timmer & Leyden, 1980). Vegetable and small fruit producers have a more efficient option: mixing inoculum of AM fungi into the horticultural potting media in which seedlings or micropropagated plantlets are grown prior to outplanting (Rai, 2001). This produces a seedling already able to take advantage of the potential benefits of mycorrhizas upon being transplanted to the field. Inoculation prior to outplanting is an option for strawberry growers, whether of bare root plants or micropropagated plantlets (Khanizadeh *et al.*, 1995; de Silva *et al.*, 1996; Gryndler *et al.*, 2002; Vestberg *et al.*, 2004).

Inocula of AM fungi are available commercially in a variety of forms. Another option for farmers, however, is to produce their own inoculum on-the-farm (Douds *et al.*, 2005). These methods, originally developed in the tropics (Sieverding, 1991; Gaur *et al.*, 2000), allow for the production of a taxonomically diverse inoculum and inclusion of locally adapted isolates in the inoculum. These characteristics are important in light of recent findings of functional diversity among AM fungi (Smith *et al.*, 2000; Stampe & Daehler, 2003) and that indigenous isolates of AM fungi can be better adapted to and more effective under local conditions than commercially available isolates (Sreenivasa, 1992; Oliveira *et al.*, 2005; Quilambo *et al.*, 2005). However, inoculum produced on-farm is not readily applied mechanically to the field, but can be efficiently mixed into horticultural potting media for the growth of plants later outplanted. Therefore, inoculum produced on-farm may be useful to vegetable and small fruit producers who grow their own plants prior to outplanting.

A field experiment was conducted to test the utilization of inoculum of AM fungi produced on-farm for strawberry production. Inoculum was produced one year, utilized to grow plants the following year, and the effect upon yield was measured the year after that (i.e. the plants' first fruit-bearing year). In addition, the influence of late season runner removal upon yield was quantified.

MATERIALS AND METHODS

Inoculum production

Inoculum was produced on-farm according to the method described in Douds *et al.* (2006) at a farm in Lititz, PA, U.S.A. Briefly, a raised bed enclosure (0.61 × 3.05 × 0.46 m) was constructed on 13 June 2003 of silt fence walls, weed barrier fabric floor, and plastic sheeting interior dividing walls to yield five 0.46 × 0.46 m compartments. The enclosure was filled to a depth of 20 cm with a 1:4 [v/v] mixture of yard clippings compost and vermiculite. Each section received ten bahiagrass (*Paspalum notatum* Flugge) seedlings, previously grown in a greenhouse for 3–4 months in 65 cm^3 conical plastic pots (pine cells, Stuewe & Sons, Corvallis, OR, U.S.A.). They were colonized by one of the following four AM fungi: *Glomus geosporum* (Nicolson & Gerdemann) Walker, *Glomus claroideum* Schenck & Smith or *Glomus etunicatum* Becker & Gerdemann, all originally isolated from The Farming Systems Trial® of The Rodale Institute, Kutztown, PA; or *Gigaspora rosea* Nicolson & Schenck (DAOM 194757). All plants in a given section were colonized by the same AM fungus. The fifth section received plants that were not colonized by AM fungi to propagate AM fungi present in soil mixed into the compost during its production (Douds *et al.*, 2006). The enclosure was weeded and watered, as needed, throughout the growing season. The bahiagrass winter killed, and the AM fungi over wintered naturally *in situ*. A pooled sample of compost and vermiculite mixture from all sections of the enclosure was collected on 24 November 2003. A most probable number bioassay (Alexander, 1965) estimated the AM fungus propagule density to be 75 propagules cm^{-3}.

Production of strawberry plants

Bare root strawberry (*Fragaria x ananassa* Duch. cv. Chandler) plants were transplanted into 50 cell flats (120 cm^3 per cell) containing a 1:19 [v/v] mixture of the AM fungus inoculum grown above in all five sections and a peat based horticultural potting mix on 20 May 2004. Each cell was estimated to contain 450 propagules of AM fungi. The potting mixture also contained 500 cm^3 of slow release fertilizer (Osmocote, 20-20-20) per 170 l, standard practice at this farm, for one set of inoculated and one set of uninoculated plants. Another set of inoculated plants was grown in potting media without the slow release fertilizer to yield three treatments initially. The two fertility regimes were included for the inoculated treatments in the event that the P level of the routine fertility practice was inhibitory to colonization of roots by AM fungi (Menge *et al.*, 1978; Linderman & Davis, 2004).

Plants were grown outdoors and watered as needed until outplanting on 30 June. A subsample of plants (six per treatment) was withheld from outplanting and characterized. Shoot dry weights, N (Wall & Gehrke, 1975), and P (Murphy & Riley, 1962) were determined. In addition, roots were cleared and stained for AM fungi (Phillips & Hayman, 1970) and analysed for percentage root length colonized by AM fungi via the gridline intersect method (Newman, 1966).

Outplanting and site characteristics

The soil was a Berks silt loam (loamy – skeletal, mixed, active, mesic Typic Dystrudepts) with pH = 6.8 and available P = 149 μg g^{-1} (Mehlich 3). The field previously had been planted to alfalfa (*Medicago sativa* L.) for five years prior to the experiment. The site was prepared first by moldboard plowing and incorporation of 22.4 metric tons of compost ha^{-1}. The compost was produced on site in a windrow with turning. The nutrient analysis of the compost was 1.7% N, 0.34% P (as P_2O_5), and 0.4% K (as K_2O) (as is basis). The compost was incorporated via roto-tilling, along with an additional 34 kg N ha^{-1} (as $(NH_4)_2SO_4$), prior to forming the beds.

Plants were outplanted into raised beds covered with black plastic: two rows per 150 m bed, 46 cm between rows on each bed and 38 cm between plants within each row. Beds were formed on 1.83 m centres. Two beds of each treatment combination were planted, and treatments alternated across the field. Irrigation was supplied under the plastic via plastic drip tape. An additional 17 kg N ha^{-1}, as $Ca(NO_3)_2$, was applied over two irrigations.

Data collection and analysis

Five plants from each treatment were unearthed on 24 August 2004. Roots were analysed for percentage root length colonized by AM fungi as above. Shoot dry weights and N and P concentrations also were quantified as above.

A second treatment regime was imposed after plants were established on 28 October 2004. The influence of removal of runners upon overall yield was examined. Alternate sections of rows were either untreated or runners were removed. Only plants previously grown with the slow release fertilizer were studied further for the impact of inoculation with AM fungi and runner management upon yield.

Harvesting began on 6 June 2005. Seven ten plant sampling units were harvested for each inoculation x runner removal treatment combination three times per week until 25 June, to yield nine harvests. Total fresh weight of fruit harvested from each ten plant unit was recorded, as well as the fresh weight of

25 randomly chosen aggregate fruit, hereafter referred to as berries. The latter measurement was used to calculate an average mass per berry.

Data were analysed via ANOVA after arcsin transformation for percentage root length colonization data. Characteristics for which significant treatment effects were found were characterized further using Tukey's Method of Multiple Comparisons ($\alpha = 0.05$).

RESULTS

Characteristics of plants at outplanting and after 8 weeks in the field

Growth of plants in flats prior to outplanting was affected by nutrient availability rather than inoculation with AM fungi (Table 1). Plants receiving the slow release fertilizer tended to have greater shoot weight and N and P concentrations than those without fertilizer. Mycorrhizas were not well developed at outplanting, and there was no indication that fertility regime affected colonization. Mycorrhizas were much more developed after 8 weeks in the field (Table 2). Inoculated plants that had received no additional fertilizer prior to outplanting tended to have the highest percentage root length colonized by AM fungi. Uninoculated plants were also colonized, indicating an active population of indigenous AM fungi. Otherwise, there were no significant differences among treatments relative to number of crowns (data not shown), shoot weight and N and P concentrations.

TABLE 1

Physical characteristics of strawberry cv. Chandler plants at time of outplanting.[z]

Treatment	Shoot dry wt. (g)	Shoot N (% dry wt.)	Shoot P (% dry wt.)	Colonization (% root length)
–M +F	2.02 a	2.77 a	0.350 a	0.0 b
+M +F	1.81 ab	2.33 a	0.314 ab	1.1 ab
+M –F	1.34 b	1.66 b	0.284 b	1.2 a
ANOVA Pr > F	0.0078	< 0.0001	0.0237	0.0201

[z]+/– M = inoculated/uninoculated with AM fungi, +/– F = with or without slow release fertilizer in the potting media. Means of six plants. Numbers in the same column with the same letter are not significantly different (Tukey's Method of Multiple Comparisons).

TABLE 2

Physical characteristics of strawberry cv. Chandler plants 8 weeks after outplanting.[z]

Treatment	Shoot dry wt. (g)	Shoot N (% dry wt).	Shoot P	Colonization (% root length)
–M +F	33.7 a	1.84 a	0.351 a	16.2 b
+M +F	31.5 a	1.92 a	0.349 a	30.5 ab
+M –F	27.1 a	1.75 a	0.317 a	42.7 a
ANOVA				
Pr > F	0.5864	0.2011	0.1756	0.0305

z +/– M = inoculated/uninoculated with AM fungi, +/– F = with or without slow release fertilizer in the potting media. Means of five plants. Numbers in the same column with the same letter are not significantly different (Tukey's Method of Multiple Comparisons).

Effect of inoculation and runner removal upon yield

Treatments exhibited similar patterns of fruit production and weight of individual fruit through the harvest period. Harvested weight of strawberries peaked at the fourth sampling, 13 June (Figure 1). Removal of runners had no effect on total yield over the harvest season (Table 3). However, the effect of prior inoculation with AM fungi significantly increased yield. Yield of inoculated plants was 17% greater than that of uninoculated plants. Mean weight per berry tended to be greater for inoculated than uninoculated plants early in the harvest period (Figure 2). Mean berry weight at the second and third harvests (8 and 10 June) was significantly greater for inoculated than uninoculated plants ($Pr > f = 0.0261$ and 0.0037, respectively). Neither inoculation nor removal of runners had a significant effect upon the average berry size over the entire sample period (Table 3). Dividing mean berry weight into total yield indicates that the increased yield of inoculated plants translated into approximately 3.6 more fruit per plant than harvested from uninoculated plants.

Economic analysis

One can estimate the economic benefit of inoculation with AM fungi in this experiment through a series of calculations. The planting scheme used here resulted in 28930 plants ha^{-1} (11760 ac^{-1}). Inoculation resulted in an average increased yield of 78.3 g $plant^{-1}$ over uninoculated controls, or 2265.2 kg ha^{-1} (Table 3). Fruit was sold per US quart (0.68 kg), for an average of $3.50 US, resulting in increased income of $11659 ha^{-1} ($4739 ac^{-1}). The inoculation required 6 cm^3 of inoculum per 120 cm^3 planting cell. Therefore, the inoculum production enclosure yielded inoculum for a maximum of 62016 plants,

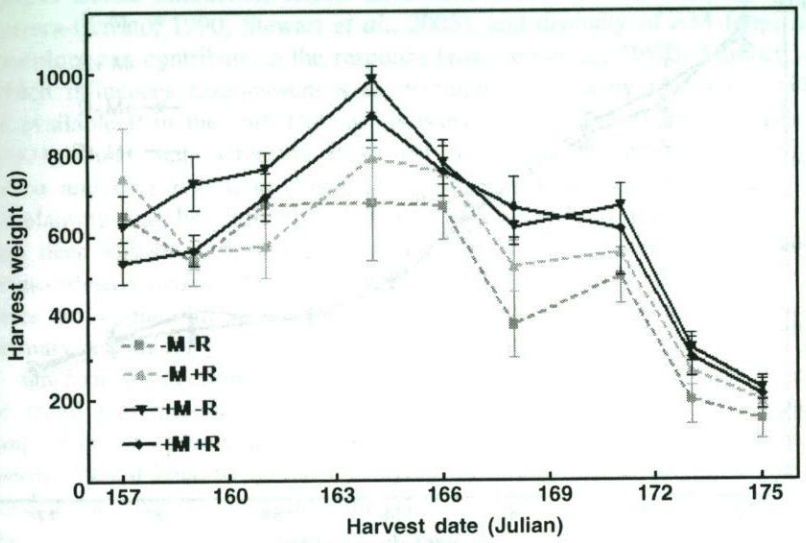

FIGURE 1. Harvested weight of strawberries. Means of seven ten plant sampling units ± SEM. + or – M = with and without inoculation with AM fungi; + or – R = plants with or without runners. Day 157 = 8 June 2005 and 175 = 25 June.

TABLE 3

Influence of inoculation with AM fungi and removal of runners upon strawberry yield and average berry weight.[z]

Treatment combination	Total yield (g 10 plants^{-1})	Wt per berry (g berry^{-1})
+M –R	5734	16.0
–M –R	4448	15.1
+M +R	5256	15.7
–M +R	4976	15.1
ANOVA	(Pr > F)	
Removal of runners	0.9464	0.7951
Mycorrhiza	0.0380	0.0627
Runners X mycorrhiza	0.1698	0.6960
(Removal of runners had no statistical effect, re-analysed after pooling)		
+M	5495 a	15.8 a
–M	4712 b	15.1 a
Pr > F	0.0372	0.0536
LSD	732	0.75

[z]+/– M = with and without inoculation with AM fungi, respectively. +/– R = with runners and without runners, respectively. Means of seven ten plant sampling units.

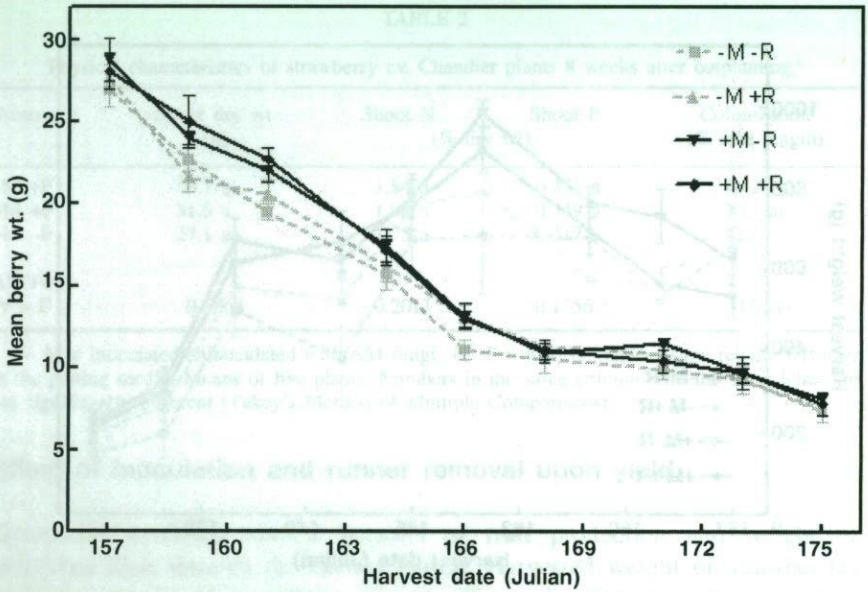

FIGURE 2. Mean weight of individual berries calculated from a random sample of 25 berries from each of seven ten plant sampling units ± SEM; + or – M = with and without inoculation with AM fungi; + or – R = plants with or without runners. Day 157 = 8 June 2005 and 175 = 25 June.

enough for 2.14 ha. The enclosure was produced at a cost of approximately $150 US. Therefore, the per ha cost was $70 for a potential return of $11659. These figures do not include the increased labour to harvest the extra fruit, the cost of the pre-inoculated bahiagrass nurse plants, or any labour charge for maintenance of the inoculum production enclosure and mixing the inoculum into the potting media.

DISCUSSION

Inoculation with AM fungus inoculum produced on-farm increased the yield of strawberry fruit by 17% over uninoculated plants. Previous work on the response of strawberry to inoculation with AM fungi produced varied results. Some showed no response in yield to inoculation (Niemi & Vestberg, 1992; Khanizadeh *et al.*, 1995; Bull *et al.*, 2005) while others have seen positive responses (Daft & Okusanya, 1973; Vosatka *et al.*, 1992; Borkowska, 2002; Sharma & Adholeya, 2004).

The variability in response of strawberry to inoculation with AM fungi may be due to several reasons common to work with other crops. First, the response can be cultivar and/or AM fungus isolate specific. Cultivar x AM

fungus isolate interaction effects have been noted for strawberry (Chávez & Ferrera-Cerrato, 1990; Stewart *et al.*, 2005), and diversity of AM fungi in the inoculum can contribute to the response (Koomen *et al.*, 1987). Another factor which influences responsiveness to inoculation for many plants is the level of available P in the soil. Daft & Okusanya (1973) and Sharma & Adholeya (2004) found that increasing P fertility decreased the response of *Fragaria vesca* and *F. x ananassa*. Lastly, it appears that inoculation at the time of outplanting may be ineffective. Inoculation of strawberry plants at outplanting has been shown to produce no more colonization of roots than found in uninoculated controls (Niemi & Vestberg, 1992; Bull *et al.*, 2005). Inoculation prior to outplanting with a taxonomically diverse inoculum likely was the primary reason for the positive yield response exhibited in this study.

On-farm production of AM fungus inoculum and utilization in vegetable or small fruit production by growers who produce their own seedlings for outplanting is a promising way to take advantage of the potential economic and environmental benefits of the AM symbiosis. Inoculum is produced one year for use the next in temperate climates, but production times can be shorter in the tropics (Sieverding, 1991). In addition to its usefulness as an amendment to horticultural potting media for seedling production, the inoculum can be applied to the field in labour-intensive farming situations such as small organic or community supported agriculture (CSA) operations. Application of the inoculum directly below seed potatoes at planting has been shown to increase yield in a high P soil (Douds *et al.*, 2007).

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